

## Research Article

# Diversity and Dynamics of Sand Flies (Diptera: Psychodidae) of Two Cutaneous Leishmaniasis Foci in the Fes-Boulemane Region of Northern Morocco

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Cutaneous leishmaniasis (CL) is an infectious disease caused by various species of *Leishmania* and transmitted by several species of sand flies (Diptera: Psychodidae). In order to evaluate the risk of leishmaniasis transmission in Fes-Boulemane, an investigation was carried out in two localities, Aichoun and Bouasseme, during 2011. From January to December, 1120 specimens were collected in Aichoun comprising six species belonging to two genera: *Phlebotomus sergenti* (76.07%), *Phlebotomus longicuspis* (9.01%), *Phlebotomus perniciosus* (8.48%), *Phlebotomus papatasi* (4.82%), *Sergentomyia minuta*, and *Sergentomyia fallax*. For Bouasseme, seven species were identified with *Phlebotomus sergenti* (60.39%) dominating, followed by *Phlebotomus perniciosus* (20%) and *Phlebotomus longicuspis* (12.15%). The remaining species, *Phlebotomus papatasi*, *Phlebotomus ariasi*, *Sergentomyia minuta*, and *Sergentomyia fallax*, were less prevalent. The activity of sand flies in both localities is marked by the dominance of *Ph. sergenti* with two peaks occurring in June and September. In order to obtain a better understanding of sand fly diversity among their species, results were analyzed by the ecological indices determinant: specific richness, the relative abundance, and Shannon-Weiner index ( $H'$ ). Further studies of sand fly diversity should employ statistical tests and molecular analyses. This study can be useful in the implementation of appropriate future control measures.

## 1. Introduction

Cutaneous leishmaniasis (CL) are parasitic diseases that remain today as a serious public health problem throughout the world. The parasitosis is caused by a "Protist Euglenobionte" that belongs to the Trypanosomatidae family, genus *Leishmania*. Three forms are observed: anthroponotic leishmaniasis caused by *Leishmania tropica* and zoonotic leishmaniasis due to *L. major* and *L. infantum* [1, 2]. *L. tropica* is the most widespread and most frequently encountered. It is transmitted by *Phlebotomus sergenti* [3].

In Morocco, there is an annual emergence of new foci of leishmaniasis. Indeed, in 2011 and 2012, the Ministry of Health recorded, respectively, 4426 and 2990 cases with 4.92% and 7.19% in the region of Fes-Boulemane [4, 5]. The first LC focus recorded from the Fes-Boulemane region was in 2001 with 1,600 cases only in the province of Moulay Yacoub [6, 7].

The objectives of our study were to identify and to make an inventory of the principle sand-flies and also to compare the specific composition and population dynamics of two biotopes located on the western and eastern slopes of the

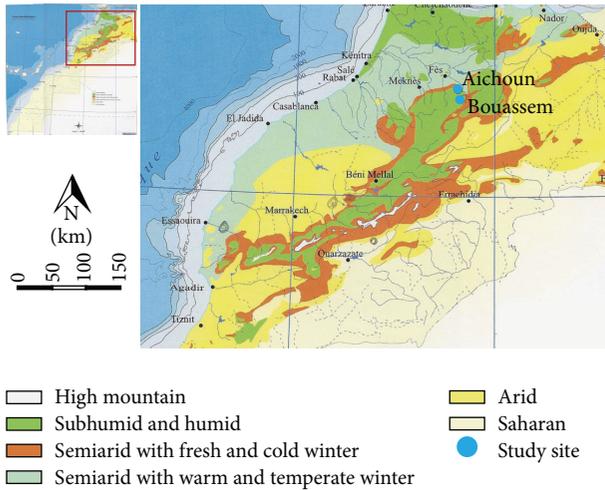


FIGURE 1: Map showing the two study site locations.

Middle Atlas, correspondingly at Aichoun and Bouasseme (Figure 1). All these steps are necessary for the surveillance and treatment of existing leishmaniasis in these two foci of Fes-Boulemane region and thus contribute to its control.

## 2. Materials and Methods

**2.1. Study Region.** Our study area covers two ecologically and epidemiologically different localities. Aichoun (33° 39'N, 04° 38'W) is located in the Northwest of the Middle Atlas; it is part of the province of Sefrou, belonging to the pastoral village territory of Tazouta with a cold semiarid climate. The average annual precipitation is 400 mm, while the temperature of the coldest month is 2°C and the maximum of the hottest month is 40°C.

From an epidemiological standpoint, 1242 cases of LC in the province of Sefrou were reported between 1997 and 2011 [8]. The majority of cases are due to *L. tropica* registered in the municipalities of El Menzel, Sefrou, Aghbalou, Tazouta Sidi Lahcen, and Ain Cheggag [9].

Bouasseme (33° 31'N, 04° 33'W) is located in the Northeast of the Middle Atlas. It is characterized by a subhumid climate receiving about 700 to 800 mm of rainfall from October to May. This town is in the province of Boulemane where the first endemic cases of leishmaniasis in *L. major* were reported [10] (Figure 1).

**2.2. Collection of Sand Flies.** Sand flies were captured once every two weeks from January to December using sticky paper traps [11]. Five stations were randomly chosen. In each station, ten traps were deposited in different biotopes (stables, barns, chicken and sheep pens, etc.) and recovered the next day. The captured sand flies are kept in airtight plastic tubes containing 70% ethyl alcohol.

The females were identified based on the examination of spermathecae, cibarium, and posterior pharynx. Male identification was mainly based on examination of the genital armature. The identification was done by using morphological identification keys [8, 12]. The morphological

differentiation between the two sympatric species *Ph. longicuspis* and *Ph. perniciosus* was made according to Berchi et al. (2007) [13]. The identification of all sand flies species was confirmed at the Laboratory of Medical Entomology in National Institute of Hygiene (the national reference laboratory in Medical Entomology).

Results are analyzed by the following ecological indices [14]:

- (i) Specific richness is the number of species that make up the stand.
- (ii) The relative abundance is the relative number of individuals of each species from all collected sand flies.
- (iii) Shannon-Weiner index ( $H'$ ) is follows:

$$H' = -\sum_{i=1}^s p_i \log p_i, \quad (1)$$

where “ $p_i$ ” can be calculated by dividing  $n_i$ , the number of species, by  $N$ , the total number of collected individuals.

## 3. Results

**3.1. Specific Composition.** The sand flies' activity period runs from April to October-November at the two sites. Catches by adhesive traps allowed harvesting 1120 sand flies of six species in Aichoun and 255 specimens belonging to seven species in Bouasseme. Five species belonged to the *Phlebotomus* genus while the other two were from the *Sergentomyia* genus.

These species are *Ph. sergenti* which is widely distributed in the two foci, *Ph. perniciosus*, *Ph. longicuspis*, *Ph. papatasi*, *Sergentomyia minuta*, and *Sergentomyia fallax*. These species are common in both localities except for *Ph. ariasi* which was collected only at Bouasseme (Figures 2 and 3). These species are considered the most abundant among the 23 species recorded in Morocco [8].

The specific richness, abundance, density, and Shannon-Weiner index ( $H'$ ) of captured sand flies are shown in Table 1. At Aichoun, the sand flies were presented with a sex ratio of 5.92. *Ph. sergenti* (Parrot 1917) was most abundant with 852 specimens (76.07%). Next was *Ph. longicuspis* (Nitzulescu 1930) with 101 specimens (9.01%), followed by *Ph. perniciosus* (Newstead 1911) with 95 specimens (8.48%). *Ph. papatasi* (Scopoli 1786) was represented by 4.82%. As for the other genus, *Sergentomyia minuta* (Rondani 1843) and *Sergentomyia fallax* (Parrot, 1921) were present in low relative abundance.

At Bouasseme, 255 sand flies were captured with species of *Phlebotomus* genus predominating both in the number of specimens captured and in their diversity (3 subgenera, 5 species). Only two species of the genus *Sergentomyia* were captured (*Sergentomyia minuta* and *Sergentomyia fallax*).

There was a difference in the diversity of the sand fly fauna between Aichoun and Bouasseme, as indicated by the values of Shannon-Weiner index ( $H'$ ). Both localities have a value of  $H'$  less than 1, with a difference of 0.1. We compared the Shannon diversity index of all the species in each study area.

TABLE 1: Percentage and the Shannon-Weiner diversity index ( $H'$ ) of the sand flies collected during the study period at both localities of the Fes-Boulemane region.

Genus	Subgenus	Species	Locality (altitude m)					
			Aichoun locality (750 m)			Bouasseme locality (1100 m)		
			R. Ab.* (%)	Shannon-Weiner index ( $H'$ )	Sex ratio	R. Ab.* (%)	Shannon-Weiner index ( $H'$ )	Sex ratio
<i>Phlebotomus</i>	<i>Larroussi</i>	<i>Ph. longicuspis</i>	9.01	0.11		12.15	0.09	
		<i>Ph. perniciosus</i>	8.48	0.13		20	0.05	
	<i>Paraphlebotomus</i>	<i>Ph. ariasi</i>	0	0.02	5.9	1.56	0	4.7
		<i>Ph. sergenti</i>	76.07	0.13		60.39	0.09	
		<i>Ph. papatasi</i>	4.82	0.03		2.74	0.08	
<i>Sergentomyia</i>	<i>S. minuta</i>	0.89	0.02		1.56	0.01		
	<i>S. fallax</i>	0.7	0.02		1.5	0.01		

\*R. Ab.: relative abundance.

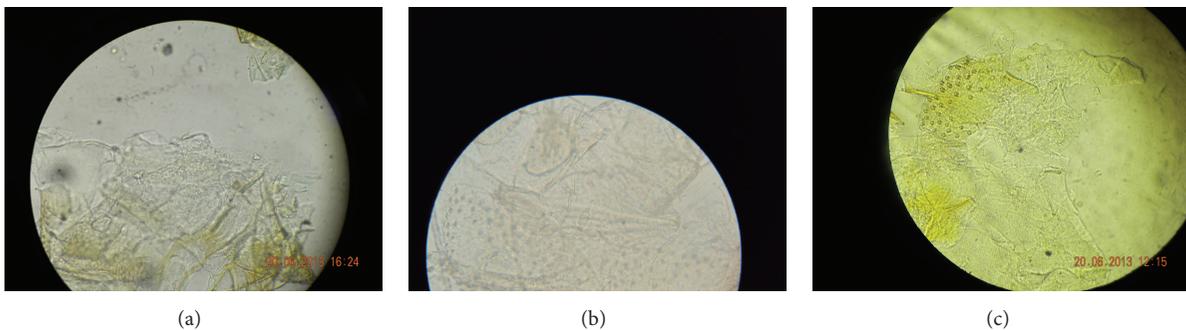


FIGURE 2: Female sand flies after dissection. ((a), (b)) Female genitalia spermathecal *Phlebotomus sergenti*; (c) Spermathecal *Phlebotomus papatasi* (personal photography;  $G \times 40$ ).

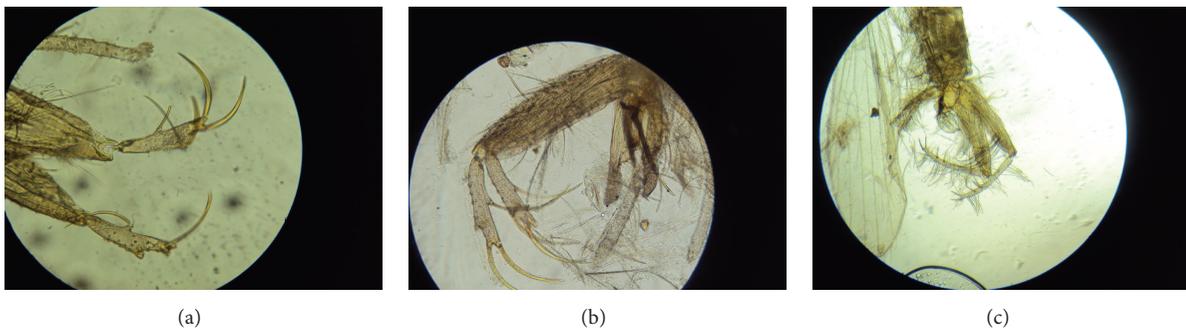


FIGURE 3: (a) Male genitalia and the extremity of penile valves in *Phlebotomus*, subgenus *Larroussi*; (b) male genitalia and the extremity of penile valves in *Phlebotomus perniciosus*; (c) male genitalia and the extremity of penile valves in *Phlebotomus papatasi* (personal photography;  $G \times 40$ ).

The values of  $H'$  at Aichoun range from 0.02 to 0.13. The most significant value was reported in *Ph. sergenti* and *Ph. longicuspis* with 0.13. But the value of this index in Bouasseme for all species varies only from 0.01 to 0.09. This difference explains why the population is more diversified at Aichoun with the predominance of *Ph. sergenti* and *Ph. longicuspis* in comparison to Bouasseme.

**3.2. Population Dynamics of Sand Flies at Aichoun and Bouasseme.** The study of the activity of sand flies in these

two areas was followed from January to December 2011. According to the capture sessions conducted in Aichoun and Bouasseme, we found a monthly evolution that varies from one species to another, biphasic in some species and monophasic in others. Throughout the study period, the activity of sand flies was marked by the dominance of *Ph. sergenti* with two peaks in June and September in both localities. In Aichoun, the activity of sand flies starts in April, coming after a gradual development observed for both *Ph. sergenti* and *Ph. longicuspis*. After June, the sand flies' activity experienced

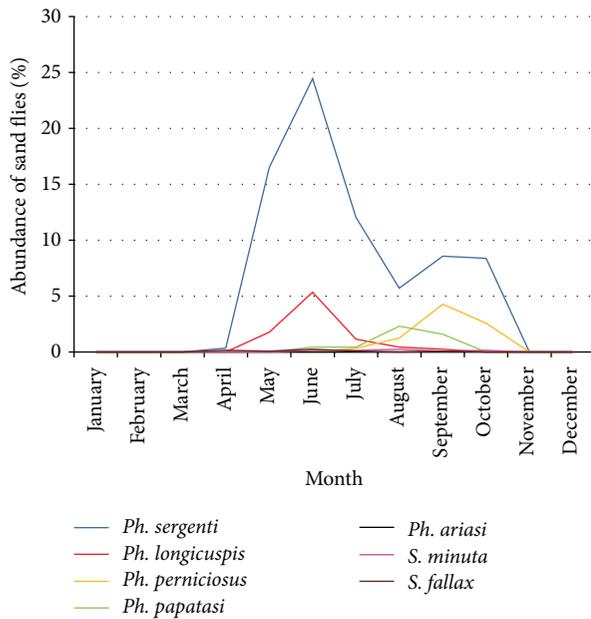


FIGURE 4: Seasonal distribution of sand flies captured at Aichoun by sticky paper traps from January to December 2011.

a fall followed by a subsequent peak for *Ph. papatasi* and then fell during the following months giving rise to an emergence of *Ph. perniciosus* with a high percentage of 4.28% in September (Figure 4). The activity of sand flies at the Aichoun locality is spread over a period of eight months stopping in November.

At Bouasseme, sand flies appeared from May to October. Compared to Aichoun, this focus has known another representation of the seasonal fluctuation of collected species. Indeed, during the period of collections, the three species *Ph. sergenti*, *Ph. perniciosus*, and *Ph. longicuspis* experienced a consistent, biphasic course with peaks, June and September, as follows: *Ph. sergenti* (26.66%, 5.88%), *Ph. longicuspis*, and *Ph. perniciosus*.

For *Ph. ariasi*, *S. minuta*, and *S. fallax*, the period of their activity was spread from June to September but with only small percentage variation (Figure 5).

#### 4. Discussion

This research is a comparative study of two leishmaniasis foci in the Fes-Boulemane region of Northern Morocco in terms of abundance, species diversity, and seasonality of sand flies. During this year long entomological survey in 2011, we collected 1,120 sand flies in Aichoun and 255 in Bouasseme, two localities of the Fes-Boulemane region. The identified species belong to two genera: *Phlebotomus* and *Sergentomyia*. Species in *Phlebotomus* genus belong to the following three subgenera: *Phlebotomus*, *Paraphlebotomus*, and *Larrousius*.

In the subgenus *Larrousius*, we reported three species: *Ph. longicuspis*, *Ph. perniciosus*, and *Ph. ariasi*. In subgenus *Paraphlebotomus*, we identified one species: *Ph. sergenti*. *Ph.*

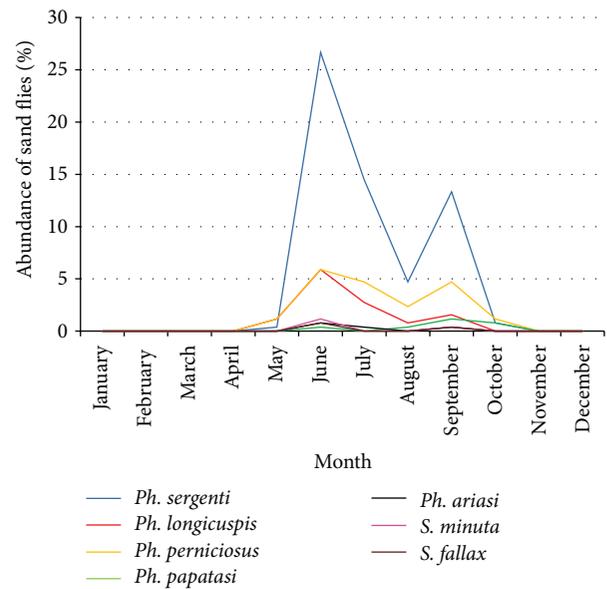


FIGURE 5: Seasonal distribution of sand flies captured at Bouasseme by adhesive traps in the period from January to December 2011.

*papatasi* was the sole species belonging to subgenus *Phlebotomus* in our collection. The two species from *Sergentomyia* (*S. minuta*, *S. fallax*) were poorly represented in sand fly fauna caught in this study.

Indeed, there is a dependency between the development of leishmaniasis and density of sand flies [15].

All proven and suspected vectors of leishmaniasis in Morocco are present in the study area: *Ph. sergenti* is confirmed as a vector of *L. tropica* anthroponotic leishmaniasis in Northern Africa, the Middle East, and Central Asia [16, 17], as well as in Morocco [18]. This species, which was also suspected to be responsible for CL in Chichaoua [19], is dominant over the other species, widely dispersed in both localities and present from May to November, with peaks of activity in June and September. This vector prefers a semiarid bioclimate, even though it was captured in all bioclimatic zones and was widespread throughout Morocco [20].

These results are in agreement with those obtained by Guessouss-Idrissi et al. [21] in the province of Taza, and El Miri et al. [22] in the province of Sidi Kacem in northern Morocco. *Ph. perniciosus* is one of the most competent vectors of *L. infantum* in Mediterranean foci [23], responsible for visceral leishmaniasis in the Northeast slope of the Rif mountains where dogs are the main reservoir of the parasite [24].

In Bouasseme, seven species were trapped. They come from two genera: *Phlebotomus* and *Sergentomyia* with *Ph. sergenti* being dominant. *Ph. ariasi* and *Ph. perniciosus*, the usual two vectors of *L. infantum* in the North of the Mediterranean [25], are also represented here. The taxonomic inventory and biodiversity sand flies obtained in Aichoun during the study period in 2011 were also obtained in 2012 [26]. This confirms continuity in the sand fly development cycle.

Similar work was carried out in the Fes-Boulemane region in a focus for cutaneous leishmaniasis at Ouled Aid in

the province of Moulay Yakoub in 2011. This study showed the same period of sand fly activity between April and November with the presence of the same species, but with different abundance [27].

From an epidemiological point of view, considering the vector-parasite relationship, we can consider *Ph. sergenti* as a potentially causative agent of *L. tropica* transmission in both Aichoun and Bouasseme bearing in mind that it is the only potential vector species of *L. tropica* in the study area. Given the low seasonality of *Ph. ariasi* and the proven role of *Ph. perniciosus* and *Ph. longicuspis* in *L. infantum* [25, 28], these two species are likely the vectors of *L. infantum* in the study area. *Ph. papatasi*, the proved vector of *L. major* in Morocco [29], has a low density at both sites; this could be explained by its preference for an arid climate [30]. The high abundance of the sand flies species in Aichoun and Bouasseme can be explained by environmental conditions, especially temperature and humidity which are conducive to a rapid biological development.

## 5. Conclusion and Perspectives

The wide distribution of sand flies and the long activity period of *Ph. sergenti*, *Ph. longicuspis*, and *Ph. perniciosus* in both foci Aichoun and Bouasseme indicate that the risk of transmission of leishmaniasis is potentially higher in this region. These results will serve as a database for further work in these two localities. They suggest the necessity for continuous entomological surveillance to lessen the risk of transmission of cutaneous leishmaniasis.

In perspective, we propose that further studies of sand fly diversity be conducted through molecular analyses, in order to positively identify sand fly species.

The determination of climatic factors favorable for sand flies development and the study of host-blood feeding preferences are also useful for developing a control program, directing operations, and evaluating their effectiveness.

## Conflict of Interests

The authors declare that they have no conflict of interests.

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